28. (New) The method of claim 27, wherein said liposome comprises a mixture of dioleoyltrimethylammonium-propane (DOTAP) and dioleoylphosphatidylethanolamine (DOPE).

29. (New) The method of claim 27, wherein said ligand comprises folate or transferrin.

30. (New) The method of claim 16, wherein said antisense nucleic acid is administered via a targeted liposome which comprises a complex of a ligand and a liposome comprising a mixture of a cationic lipid and a neutral lipid.

31. (New) The method of claim 30, wherein said liposome comprises a mixture of dioleoyltrimethylammonium-propane (DOTAP) and dioleoylphosphatidylethanolamine (DOPE).

32. (New) The method of claim 30, wherein said ligand comprises folate or transferrin.

REMARKS

Support for new claims 21-32 can be found, for example, on page 14 of the specification. No new matter is introduced through these new claims.

Claims 1-6 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-4 of U.S. Patent 6,027,892. Enclosed herewith is a terminal disclaimer signed by the Applicants' undersigned representative.

Claims 1 and 2 have been rejected under 35 U.S.C. §101 on the basis that the sequence which is the focus of the claims is

not identified as being an isolated sequence. Applicants respectfully submit that this rejection has been obviated by the amendment above to claim 1. Claim 1 now provides that the sequence is an isolated sequence.

Claims 3-5, 7, 8, 12-14, 16 and 17 have been rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification as filed teaches only one specific example of a HER-2 antisense sequence and so does not provide a representative number of species of HER-2 for the claimed functions of reducing radiation or drug resistance in any cell or person. The examiner asserted that one of ordinary skill in the art could not immediately envisage a representative number of species of HER-2 antisense having the claimed functions since the functions of antisense in cells in a whole organism were taught in the art to be sequence specific. This rejection is traversed.

A great deal of work has been done in the field of antisense oligonucleotides as therapeutic agents in recent years. The examiner has made comments as part of this rejection and elsewhere in the Action as to the unpredictable activity of antisense oligos. Applicants respectfully submit that there now is, in fact, significant predictability in this field. Although the effectiveness of an oligo designed randomly may not be predictable, there are guidelines recognized in the art that significantly simplify oligo selection. Applicants have discussed three significant guidelines on page 8 of their application. For example, it has been recognized in the art that oligos substantially complementary to an RNA sequence at or near the initiation codon of the gene of interest typically are specific to the gene. It also has been found that oligos complementary to an RNA sequence around the promoter sequence or at single stranded loops typically are specific to that gene.

Thus, Applicants give a significant amount of guidance in their application regarding useful HER-2 antisense oligonucleotides. In addition to the guidelines set forth above, they further teach that the oligo preferably comprises a sequence of at least 8 nucleotides but not more than about 40 nucleotides and more preferably comprises between about 15 and 20 nucleotides. Finally, Applicants illustrate the usefulness of a specific oligo identified in the application as SEQ ID NO:3. In view of all these teachings, it would not be difficult for one of skill in the art to construct an oligo useful in the method of this invention.

Enclosed herewith is a Declaration by one of the named inventors, Dr. Esther Chang. In the declaration she states that, in addition to the antisense oligo identified in the application as SEQ ID NO:3, her laboratory has made five other antisense oligonucleotides specific to HER-2. This is further evidence that no more than routine experimentation is required by one of ordinary skill in the art to make and select oligonucleotides useful in the method of the claimed invention.

Thus, contrary to the examiner's assertions, one of skill in the art would recognize that Applicants' specification provided adequate guidance for the selection of useful oligos.

Claims 3-8, 11-17 and 20 have been rejected under 35 U.S.C. § 112, first paragraph, on the basis that although the specification is enabling for the methods claimed in U.S. Patent 6,027,892 and the use of SEQ ID NO:3 in carcinoma cells in mice, it does not provide enablement for the methods of making and using any HER-2 antisense in any species for the breadth of methods claimed for use *in vivo*. The examiner asserted that there is a high level of unpredictability in the antisense art for therapeutic, *in vivo* use. This rejection is traversed.

The examiner cited a number of references as support for her assertion that there is great uncertainty regarding the therapeutic use of antisense oligos. She began by quoting a paper by Ma et al. that such oligos have to have certain characteristics, including resistance to degradation, adequate bioavailability, the ability to hybridize specifically with the target nucleic acid, and nontoxicity. Applicants submit that this list is simply that, a list of characteristics. It is not a list of obstacles that have yet to be overcome. There currently are at least 46 antisense oligonucleotide clinical trials for various diseases, 10 of which are in Phase III and 20 of which are in Phase II, and one antisense therapeutic product, Vitravene ®, has been approved by the FDA. As discussed above, scientists have developed a number of guidelines to simplify the process of obtaining antisense oligonucleotides that are specific for a desired target. They also have found ways to modify antisense oligos so as to increase their stability and cellular uptake. Such modifications, such as modifying the backbone or sugar components of the oligo, are well-known to persons of skill in the art and are discussed in the current application at page 9.

The examiner also cited a statement in a paper by Jen et al. that although there have been a number of Phase I and II trials using antisense oligonucleotides, "virtually all have been characterized by a lack of toxicity but only modest clinical effects." The first part of this assessment is accurate and, obviously, desirable. Although the second part of this statement may have been accurate at some point in the past, it is not accurate today. Significant clinical effects are being seen in all of oligos currently in Phase III clinical trials and many of those in Phase II trials, and the FDA-approved antisense oligo product has been shown to be highly effective in treating CMV retinitis in patients with AIDS.

The examiner further cited a statement by Green et al. that "the future of nucleic acid therapeutics using antisense ODNs ultimately depends on overcoming the problems of potency, stability, and toxicity; the complexity of these tasks should now be apparent." Applicants submit that although potency, stability and toxicity are factors that must be considered in developing an antisense oligo useful as a therapeutic agent, none of these factors presents such a challenge that undue experimentation is required to overcome it. As noted above, there are clear guidelines known in the art for making an oligo that has the desired specificity, and specificity can be determined through such routine tests as Western blot analysis and XTT cytotoxity assays. The stability of an oligo can be improved through a variety of modifications to the backbone or sugars of the molecule, as discussed above and in the application. Toxicity can be tested *in vitro*. Green et al. noted on page 98 of their paper that "numerous reports have demonstrated the application of these [antisense] molecules in the study of biologic processes and their ability to function as intended" and "[a]ntisense ODNs targeting a number of distinct oncogenes have been shown to inhibit tumor growth in experimental models in vitro and in vivo." Green et al. go on to describe the beneficial results observed in a number of clinical trials ongoing in 2000, when their paper was published. These trials focused on the use of antisense oligos to treat tumors, vascular disease, inflammatory bowel disease and antiviral therapy. The beneficial results seen in 2000 have since been verified. Some free oligos have very short half-lives in the body, and as a result are being administered continuously via a pump for an extended period of time to achieve therapeutic efficacy. Although such a form of administration may not be optimum for patient comfort, it does provide an efficacious way to provide treatment. The fact that

there remain some questions about how best to optimize the delivery of antisense oligos does not mean that such oligos are not useful therapeutic tools nor that Applicants have not enabled their invention.

The examiner made reference to a list of outstanding questions noted in the Agrawal et al. reference cited in the Office Action. In doing so, however, she ignored the overall theme and tenor of the paper; as the authors state in the Abstract:

> Antisense oligonucleotides provide a simple and efficient approach for developing target-selective drugs because they can modulate gene expression sequence-specifically.

It also is worth noting that several of the papers relied upon by the examiner were published in 1996-1998. Applicants respectfully submit that the teachings of these papers were out of date by the time the Applicants filed their application. This field has garnered a significant amount of attention and has developed rapidly, as evidenced by the number of products in Phase II and III clinical trials. Additional improvements, such as further modifications to the antisense backbone, for example, may be developed, but the premise underlying the administration of antisense oligos is sound and was workable at the time the Applicants filed their application. The effectiveness of these types of modifications is evidenced by the number of free oligos which are the subject of clinical trials.

Further, Applicants note that a majority of their claims are directed to the administration of antisense oligos encapsulated within targeted liposomes. By encapsulating the antisense oligo within a liposome and linking the resulting complex to a natural ligand of the target cell, cell-specific antibodies or synthetic

ligands that will bind to the target cell, increased selectivity for the cell type of interest can be achieved. The liposomes have long circulating half lives and provide increased stability, intracellular uptake and biological activity, as evidenced, for example, in Figures 8 and 9 of the application. They have an ability to localize in high concentrations in solid tumors, thus making them very useful for treatment of cancers. The tumor-targeting facet and receptor-mediated endocytosis associated with the ligand enables the therapeutic oligo to be introduced efficiently and specifically to the tumor cells *in vivo* as well as *in vitro*.

Applicants have demonstrated in their application that targeted liposomes in accordance with their invention do indeed target tumors to the exclusion of normal organs and tissues. As they report on page 15 of their application, intravenous administration of complexes comprising the β-galactosidase reported gene encapsulated in liposomes comprising a mixture of DOTAP and DOPE and complexed to either folate or transferrin were found to transfect breast, prostate and SCCHN tumors with an efficiency of between about 50 - 70%, while normal tissues and organs showed no sign of reported gene expression. Even micrometastases in the lung, spleen and lymph nodes showed evidence of efficient and specific transfection. The targeted liposome delivery system of claims 5, 14 and 21-32 thus clearly provides an efficient way to deliver an antisense oligo to specific cells of interest. The art recognizes that liposomes present a good delivery system for antisense oligonucleotides; the present targeted liposomes enhances the usefulness of such a delivery system.

The examiner agreed in the Office Action that the present application shows that the particular antisense oligo identified in the application as SEQ ID NO:3 could be administered via

targeted liposomes for certain types of cancer but asserted that trial and error would be necessary to design regimens for administration of any other antisense in any liposome. Applicants respectfully submit that this is not correct. Applicants have shown that a variety of ligand-liposome complexes can be used to administer antisense oligos to several different types of tumor cells. No evidence has been provided that such complexes cannot be used effectively to administer other antisense oligonucleotides that have been shown through *in vitro* studies to be specific for target cells of interest. As Applicants have discussed above, it is within the abilities of one of skill in the art to determine whether a particular antisense oligo is specific for a gene of interest. Once this has been determined, that oligo can be administered to a patient via ligand-liposome complexes such as described and claimed in the application.

The examiner has asserted that the experiments described in the application, in which an antisense oligo was administered to mice, are not sufficient to enable the administration of antisense oligonucleotides to other mammals, such as humans, as the art does not recognize mouse models as necessarily predictive of results in humans. She cited references by Blackshear et al. and by Sigmund et al. as support for her conclusion.

Applicants respectfully submit that the examiner's conclusion regarding the suitability of the mouse as a model for human treatment is over-reaching and, therefore, inaccurate. Contrary to the examiner's assertions, the nude mouse is an accepted model in the development of human cancer therapy. The examiner appears to have overlooked that although the *in vivo* experiments were carried out in mice, the mice were ones in which xenografts, i.e., human tumors, had been induced. The use of

xenograft-induced mice is, in fact, the standard model in the field of cancer treatment. Applicants respectfully direct the examiner's attention to the website for the National Cancer Institute, an entire section of which focuses on mouse models. The NCI has a collaborative program, the NCI Mouse Models of Human Cancers Consortium, (MMHCC), and sponsors a variety of other projects to "develop, analyze and apply mouse cancer models." See the first page of the "e-mouse" portion of the NCI website, a copy of which is attached. Also attached is the first page of the "Mouse Models" subsection of that portion of the site. The examiner's reliance upon the Blackshear et al. reference, which focused on animal models with spontaneous or chemically induced mammary gland carcinogenesis, rather than on mice in which human tumors had been induced, thus is misplaced.

Similarly, the reference by Sigmund et al., focuses on transgene, knock-out and gene-targeted models and problems associated with these models. Applicants respectfully submit that this reference is irrelevant; again, their own experiments were conducted using in-bred mice carrying human tumors. As such, they are very different from those discussed by Sigmund et al.

Applicants thus respectfully submit that their disclosures are sufficient to show one of skill in the art that antisense molecules shown to have a desired specificity can be administered to humans as therapeutic agents. Any experimentation required would be routine; specificity can be determined through routine tests such as Western blot analysis and XTT cytotoxicity assays, and there are known guidelines for modifying antisense sequences to improve their stability and cellular uptake if that appears desirable. An oligo shown to target a sequence and to affect protein expression in cell culture will have that same activity *in vivo*. Further, such oligos can be delivered *in vivo* either

through administration of free oligos or via liposomes, as evidenced by ongoing clinical trials. A preferred delivery system is the targeted liposomes disclosed and claimed in the present application.

In view of the foregoing amendments and discussion, Applicants respectfully submit that the pending claims are in condition for allowance.

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Organ Site Model Overview

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- Nervous System Cancer Models
- Ovarian Cancer Models
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- Skin Cancer and Melanoma Models
- Cervical Cancer Models
- Oral Cancer Models
- Sarcoma Cancer Models

Therapeutics Links
Includes links to general information about specific therapies, models and genetics regarding resistance. Information about clinical trials in humans, how to design a mouse clinical trial, categories of therapeutic compounds and genetic modifiers/strain differences.

Mouse Models of Cancer Publications
PubMed links for mouse models of cancer publications by cancer type.

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Information about interactions between the MMHCC and Pharma/Biotech companies; includes the legal issues about patents, licensing and funding opportunities.

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Mouse models that recapitulate many aspects of the genesis, progression, and clinical course of human cancers are valuable resources to cancer researchers engaged in a variety of basic, translational, clinical, and epidemiological investigations. The **NCI Mouse Models of Human Cancers Consortium (MMHCC)** is a collaborative program designed to derive and characterize mouse models, and to generate resources, information, and innovative approaches to the application of mouse models in cancer research.

In addition to the MMHCC initiative, the NCI sponsors numerous other projects to develop, analyze, and apply mouse cancer models. This site is intended to provide the cancer research community with information about mouse models and mouse research generated by the MMHCC and other NCI-supported projects.

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Overviews of organ site models, mouse model publications, industrial partners, and therapeutics

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Provides "how to" information for mouse model engineering, which includes tutorials about statistics and histology (comparison of mouse models and human disease, images, markers, and protocols). Online books, journals, dictionary, cancer news, mouse strain nomenclature

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